

## CLAIMS

1. A method of producing a non-bovine pre-prochymosin, prochymosin or chymosin, the method comprising the steps of

5

(i) isolating or constructing a nucleic acid sequence coding for the pre-prochymosin, prochymosin or chymosin,

(ii) constructing an expression vector comprising said coding sequence and, operably

10 linked thereto, appropriate expression signals permitting the pre-prochymosin, prochymosin or chymosin to be expressed in a host cell,

(iii) transforming said host cell with the expression vector,

15 (iv) cultivating the thus transformed host cell under conditions where the coding sequence is expressed, and

(v) harvesting the pre-prochymosin, prochymosin or chymosin.

20 2. A method according to claim 1 wherein the coding sequence is derived from a mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species, a porcine species, an *Equidae* species and a primate species.

3. A method according to claim 2 wherein the ruminant species is selected from the group  
25 consisting of deer species, buffalo species, antelope species, giraffe species, ovine species and caprine species.

4. A method according to claim 1 wherein the coding sequence for pre-prochymosin, prochymosin and chymosin is isolated or derived from *Camelus dromedarius*.

30

5. A method according to claim 1 wherein the nucleic acid sequence codes for a fusion protein comprising pre-prochymosin, prochymosin or chymosin.

6. A method according to claim 5 wherein the fusion protein comprises glucoamylase or a  
35 fragment thereof.

7. A method according to any of claims 1-6 wherein the pre-prochymosin, prochymosin or chymosin, or a fusion protein thereof, is secreted over the host cell membrane.

8. A method according to claim 1 wherein the expression vector is derived from pGAMpR  
5 as described in Ward et al., 1990 by substituting the coding sequence of that vector for bovine prochymosin with a coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

9. A method according to claim 8 wherein the expression vector is pGAMpR-C as con-  
10 tained in the *Aspergillus niger* var. *awamori* strains deposited under the accession numbers CBS 108915 and CBS 108916.

10. A method according to any of claims 1-9 wherein the transformed host cell is selected  
15 from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a mammalian cell, an insect cell and a plant cell.

11. A method according to claim 10 wherein the host cell is *Aspergillus niger* var. *awamori*.

12. A method according to claim 11 wherein the *Aspergillus niger* var. *awamori* host cell is  
20 selected from the group consisting of CBS 108915 and CBS 108916.

13. A method according to any of claims 1-12 wherein the yield of pre-prochymosin, pro-  
chymosin or chymosin milk clotting activity is at least 25% higher than the yield of bovine  
25 pre-prochymosin, prochymosin or chymosin milk clotting activity obtained when using, under identical production conditions, the same expression vector, but with a coding sequence for bovine pre-prochymosin, prochymosin or chymosin in place of the coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

14. A method according to any of claims 1-13 comprising, as a further step, that the har-  
30 vested pre-prochymosin, prochymosin or chymosin is subjected to a deglycosylation treatment.

15. A method according to claim 1 wherein the host cell is a cell expressing a deglyco-  
35 sylating enzyme.

16. A method according to claim 15 wherein the deglycosylating enzyme is endoH.

17. A DNA construct capable of expressing non-bovine pre-prochymosin, prochymosin or chymosin, said construct comprising an expression vector comprising a nucleic acid sequence comprising a gene coding for the pre-prochymosin, prochymosin or chymosin and, operably linked thereto, appropriate expression signals permitting the pre-prochymosin, prochymosin or chymosin to be expressed in a host cell.

18. A DNA construct according to claim 17 where the construct comprises a sequence coding for a signal peptide for the pre-prochymosin, prochymosin or chymosin.

19. A DNA construct according to claim 17 where the expression signal comprises a promoter not natively associated with the coding sequence.

20. A DNA construct according to claim 17 where the coding sequence is derived from a mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species, a porcine species, an *Equidae* species and a primate species.

21. A DNA construct according to claim 20 where the ruminant species is selected from the group consisting of deer species, buffalo species, antelope species, giraffe species, ovine species and caprine species.

22. A DNA construct according to claim 20 where the coding sequence is derived from *Camelus dromedarius*.

23. A DNA construct according to any of claims 17-22 where the nucleic acid sequence codes for a fusion protein comprising the pre-prochymosin, prochymosin or chymosin.

24. A DNA construct according to claim 23 where the fusion protein comprises glucoamylase or a fragment thereof.

25. A DNA construct according to any of claims 17-24 where the expression vector is derived from pGAMpR as described in Ward et al., 1990 by substituting the coding sequence of that vector for bovine prochymosin with a coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

26. A DNA construct according to claim 25 where the expression vector is pGAMpR-C as contained in the *Aspergillus niger* var. *awamori* strains deposited under the accession numbers CBS 108915 and CBS 108916.

5 27. A DNA construct according to claim 17 comprising a sequence coding for a deglycosylating enzyme.

28. A DNA construct according to claim 27 where the deglycosylating enzyme is endoH.

10 29. A DNA construct according to any of claims 17-28 where the coding sequence is a naturally occurring coding sequence.

30. A DNA construct according to any of claims 17-28 where the coding sequence is derived from a naturally occurring coding sequence by one or more silent nucleotide substitution(s).  
15

31. A host cell transformed with the DNA construct according to any of claims 17-30.

32. A host cell according to claim 31 which is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a mammalian cell, an insect cell and a plant cell.  
20

33. A host cell according to claim 32 which is of *Aspergillus niger* var. *awamori*.

34. A host cell according to claim 33 where the *Aspergillus niger* var. *awamori* host cell is selected from the group consisting of the strains deposited as CBS 108915 and CBS 108916.  
25

35. A composition comprising a non-bovine pre-prochymosin, prochymosin or chymosin produced by the method of any of claims 1-16.

30

36. A composition according to claim 35 where the pre-prochymosin, prochymosin or chymosin is in a substantially deglycosylated form.

37. A composition according to claim 35 or 36 comprising pre-prochymosin, prochymosin or chymosin derived from the group consisting of a *Camelidae* species, a buffalo species, an ovine species or a caprine species.

5 38. A method of manufacturing cheese, comprising adding a milk clotting effective amount of the composition according to claim any of claims 35-37 to milk and carrying out appropriate further cheese manufacturing steps.

39. A method according to claim 38 wherein the milk is selected from the group consisting  
10 of cow's milk, camel's milk, buffalo milk, goat's milk, sheep's milk and a mixture of any such milk types.

40. A method according to claim 38 wherein the yield of cheese obtained is higher than the yield obtained under identical manufacturing conditions using the same amount of bo-  
15 vine pre-prochymosin, prochymosin or chymosin.

41. A method of manufacturing cheese, comprising adding a milk clotting effective amount of a non-bovine prochymosin or chymosin to the milk and carrying out appropriate further cheese manufacturing steps, the non-bovine prochymosin or chymosin having in said milk  
20 a C/P ratio in the range of 2-20.

42. A method according to claim 41 wherein the milk is cow's milk.

43. A method according to claim 41 or 42 wherein the non-bovine prochymosin or chymo-  
25 sin is derived from *Camelus dromedarius*.

44. A milk clotting composition comprising a milk clotting bovine enzyme selected from prochymosin, chymosin and pepsin and a non-bovine milk clotting enzyme selected from prochymosin, chymosin, pepsin and a microbial aspartic protease.

30

45. A composition according to claim 44 where the milk clotting activity ratio between the bovine and the non-bovine milk clotting enzyme is in the range of 1:99 to 99:1.

46. A composition according to claim 44 or 45 where the non-bovine milk clotting enzyme  
35 is derived from *Camelus dromedarius*.

47. A method of manufacturing cheese from milk, comprising adding a milk clotting effective amount of a composition according to any of claims 44-46 to the milk and carrying out appropriate further cheese manufacturing steps.

5 48. A method according to claim 47 wherein the milk is cow's milk.

Patent application/P10039US01/CM/LJz/05112001